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Effect of extracellular polymeric substances on sludge reduction in the humic-activated sludge anoxic-oxic-settling-anaerobic process

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ABSTRACT

The wide utilization of the activated sludge process for treating domestic wastewater has resulted in the huge production of excess sludge, posing a serious disposal problem. In the present study, a humic-activated sludge-anoxic-oxic-settling-anaerobic (HS-A-OSA) process was used to study the function of humic activated sludge on the efficiency of sludge reduction. For this purpose, two anoxic-oxic-settling-anaerobic (A-OSA) experimental devices were fabricated. Between the two devices, one device with the composite humic biological filler served as the HS-A-OSA process and the other device served as the comparison. The processes were operated with a mixed liquor suspended solids concentration of 3,000 mg/L for a period of 60 days. The sludge internal recycling was 100% from the sedimentation basin to the anaerobic sludge reaction basin, followed by the anoxic basin. The return sludge residence time was 5.5 h with both devices. During the operation period, 23.2% of excess sludge reduction was observed. The chemical oxygen demand and total nitrogen removal efficiencies improved in the HS-A-OSA process. Based on the variations in polysaccharide, protein, humic acid and extracellular polymeric substances concentrations in different basins, the main reason for improved sludge reduction was that the sludge disintegration velocity of the HS-A-OSA process was higher than the A-OSA process.

Keywords: HS-A-OSA process; Sludge reduction; Extracellular polymeric substances; Polysaccharide; Protein; Humic acid

1. Introduction

The activated sludge process is most widely used for treating domestic wastewater. A great amount of activated sludge is generated during the operation, part of which should be withdrawn and disposed to maintain the appropriate level of biomass concentration in the aeration basin [1, 2]. The expense for the disposal of the excess sludge accounts for about 50% to 60% of the total cost in a wastewater treatment plant [3, 4]. Therefore, sludge reduction technologies have become an urgent concern in the field of wastewater treatment [5–7]. The anoxic-oxic-settling-anaerobic (A-OSA) process is an economical treatment. The A-OSA process is a modification of the anoxic/oxic (A/O) process, in which an anaerobic sludge basin is inserted into the return sludge system of a secondary sedimentation basin. The activated sludge is in the anoxic/oxic alternate environment. Adenosine triphosphate (ATP), which is produced in the oxic basin by microorganisms, is not used for making cells, but as an energy source of microorganism life activities in the anaerobic basin. When the sludge comes back to the oxic basin with adequate nutrition, ATP is reproduced and used for microorganism metabolism in the anaerobic basin. Anabolism and catabolism of

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microorganisms is uncoupled. Sludge production is reduced with good wastewater treatment efficiency [8]. Lu Yanhua [9] reported that sludge production of A-OSA process is 30.6% lower than the A/O process, but total nitrogen (TN) removal efficiency is 82.8% and less than the A/O process. To improve the nitrogen removal efficiency of A-OSA, humic-activated sludge is known for high microbial activity and capacity of nitrogen and phosphorus removals [10–12]. The humic-activated sludge A-OSA (HS-A-OSA) process can be built with a composite humic biological filler placed inside the oxic basin of the A-OSA process.

Eighty percent of activated sludge dry weight is comprised of extracellular polymeric substances (EPS) [13]. EPS are an important component of activated sludge. The components of EPS are similar to the cellular content of microorganisms in activated sludge. EPS mainly includes a number of neutral and acid polymers, such as protein, polysaccharides, and humic acid. Indeed, the prime organic components in EPS are protein, polysaccharides, and humic acid. EPS generates a series of reactions, such as polymerization, dissociation, and degradation in the anoxic/oxic alternate environment. When the sludge is in an anaerobic environment with nutrient deficiencies, EPS is decomposed as nutrient in anaerobic metabolism for activated sludge microorganisms, which makes a great contribution in sludge reduction [14–15]. The present study utilizes the advantages of humic-activated sludge and the A-OSA process for sludge reduction and the capacity of nitrogen and phosphorus removal. The objectives of this study were to evaluate the sludge reduction efficiency and the mechanism underlying the HS-A-OSA process and variations in EPS characteristics of humic-activated sludge in different reaction basins. The performance of the HS-A-OSA process was evaluated by comparing the HS-A-OSA process with the A-OSA process.

2. Materials and methods

2.1. Experimental devices

Two different experimental devices were fabricated. The first device (Fig. 1) is the HS-A-OSA process, and consists of an anoxic basin, an oxic basin with the composite humic biological filler, a sedimentation basin, and an anaerobic sludge reaction basin. The filler was placed into a cage inside the oxic basin. The second experimental device (Fig. 2) is the A-OSA process, which is for comparison. The second experimental device consists of an anoxic basin, an oxic basin, a sedimentation basin, and an anaerobic sludge reaction basin.

The devices were made of organic glass. The length, width, and height of the anoxic basin was 20 cm, 20 cm, and 35cm, respectively. The effective volume of the anoxic basin was 14 L. The length, width, and height of the oxic basin was 60 cm, 20 cm, and 35 cm, respectively. The effective volume of the oxic basin was 42 L. The sedimentation basin was a cylinder with a truncated cone-shaped mud bucket in the bottom of the reactor. The diameter of the sedimentation basin was 30 cm, height was 40 cm, and the effective volume was 28 L. The anaerobic sludge reaction basin was a cylinder. The diameter was of the anaerobic sludge reaction basin was 40 cm, the height was 40 cm, and the effective volume was 50 L.

The materials of the composite humic biological filler in the oxic basin were residual activated sludge, humus, clay, and silicon compounds. The filler was a taupe cylinder. The average dimensions and related parameters of the filler were as follows: diameter, 20 mm; length, 30 mm; volume, 9.5 cm^3; weight, 15.3 g; density, 1.6 g/cm^3; surface area, 9.51 m^2/g; total pore volume, 0.05 cc/g; and pore size, 17.6 A.

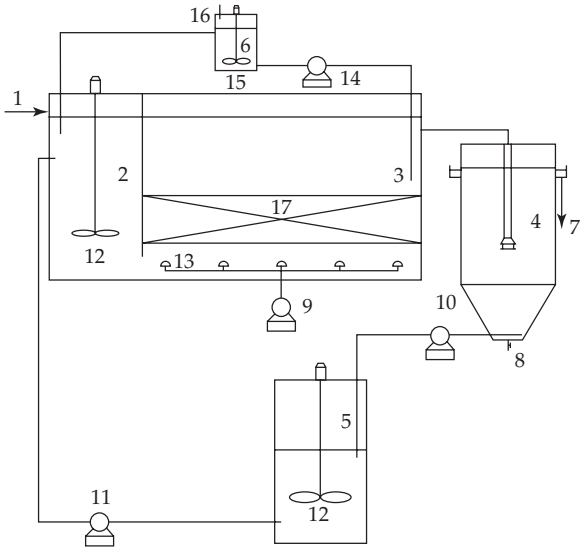


Fig. 1. Experimental device of the HS-A-OSA process.
Notes: 1—influent, 2—anoxic basin, 3—oxic basin, 4—sedimentation basin, 5—anaerobic sludge reaction basin, 6—closed vessel, 7—effluent, 8—mud pipe, 9—air blower, 10—return sludge, 11—return anaerobic sludge, 12—mechanical stirrer, 13—aerator, 14—nitrification liquid internal recycle, 15—mechanical stirrer, 16—exhaust pipe and 17—composite humic biological filler.

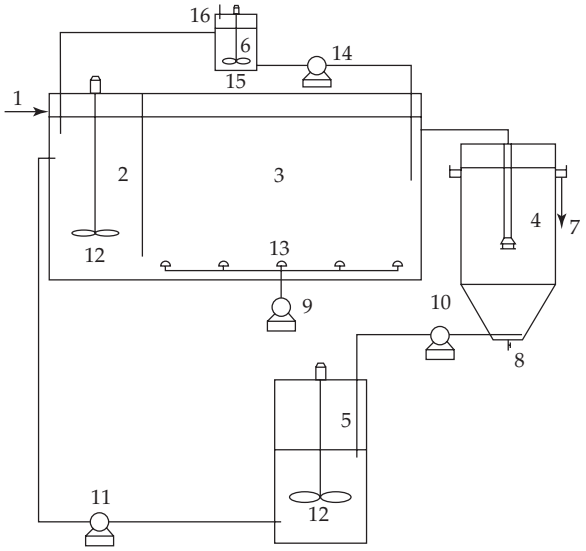


Fig. 2. Experimental device of the A-OSA process.
Notes: 1—influent, 2—anoxic basin, 3—oxic basin, 4—sedimentation basin, 5—anaerobic sludge reaction basin, 6—closed vessel, 7—effluent, 8—mud pipe, 9—air blower, 10—return sludge, 11—return anaerobic sludge, 12—mechanical stirrer, 13—aerator, 14—nitrification liquid internal recycle, 15—mechanical stirrer and 16—exhaust pipe.

2.2. Experimental conditions

Synthetic domestic wastewater was used as the experiment influent by adding peptone and $C_6H_{12}O_6$, $(NH_4)SO_4$, and KH_2PO_4 as the source of carbon, nitrogen and phosphorus, respectively, and adding $NaHCO_3$ to adjust the pH of the influent. The pH was controlled to 7.0–7.5, the chemical oxygen demand (COD) was 400–420 mg/L, the TN was 50–60 mg/L, the ammonia nitrogen (NH_4-N^+) was 48–55 mg/L, the total phosphorus (TP) was 6–7 mg/L, the mixed liquid suspended solids (MLSS) was 3000 mg/L, and the temperature was approximately 25°C. The inflow of each device was 110 mL/min. The hydraulic retention time of the anoxic and oxic basins was 2.12 h and 6.36 h, respectively. The oxic basins used blast aeration, with the air compressors providing blasts of compressed air through porous stones. Dissolved oxygen (DO) was 2–3 mg/L. At the bottom of the oxic basin, a nitrification liquid internal recycling pipe connected with a closed vessel and an anoxic basin was used as a peristaltic pump. The diameter was 10 cm, the height was 15 cm, and the effective volume was 1.2 L. The hydraulic retention time of the nitrification liquid was 5 min in the closed vessel. The closed vessel with a blender and exhaust pile was used for degassing to maintain anoxic condition; the nitrification liquid internal recycling was 200% from oxic basin to anoxic basin [2]. At the bottom of sedimentation basin, a sludge internal recycling pipe connected with the anaerobic sludge reaction basin and anoxic basin using a peristaltic pump. The sludge internal recycling was 100% from sedimentation basin to anoxic basin. The residence time of return sludge was 5.5 h in the anaerobic sludge reaction basin. The sludge retention time of the two experimental devices was 12 days.

The experimental sludge was taken from a sewage treatment plant in the city of Changchun. Two experimental devices were seeded for cultivation and domestication under the same laboratory conditions. When MLSS was >3,000 mg/L, excess sludge was withdrawn.

2.3. Analytical methods

The MLSS, sludge volume index (SVI), COD, and TN of the influent and effluent were analyzed following methods detailed in *Monitor Analysis Methods of Water and Wastewater* (4th edition) [16]. The microorganism morphologies in both experimental devices were detected with a FESEM (Quanta-200: FEI, Hillsboro, OR, USA). The analysis of EPS in biomass was made through a centrifugal extraction method. The mixed liquor of different basins was centrifuged (8000 *g* for 30 min at 4°C) to separate the soluble EPS (SEPS) from bound EPS (BEPS). After collecting SEPS, the remaining solid was washed and re-suspended in light saline water (1.0% NaCl solution). The extracted solution was then separated from the sludge solids by ultrasonication and centrifugation (8,000 *g* for 10 min). The supernatant obtained at this stage was referred to as the loosely BEPS solution. After collecting the loosely BEPS, the remaining solid was washed and re-suspended in light saline water again. The extracted solution was separated from the sludge solids in a water bath (100°C) and centrifugation (12,000 *g* for 15 min), the supernatant obtained at this stage was referred to as the closely BEPS solution. The sum of the loosely BEPS and bound EPS represented the total BEPS content. The EPS solution was then measured in terms of polysaccharide, protein, and humic acid contents using the anthracenone-sulfuric acid method with glucose as a standard and the modified Folin–Lowry method with bovine serum albumin and humic acid as standards, respectively [17–20]. The sum of polysaccharide, protein, and humic acid represented the total EPS content.

3. Results and discussion

3.1. Sludge reduction and wastewater treatment efficiency

Calculation of daily sludge production (DSP_{day}) in both the experimental devices, and comparison of the data will provide an accurate estimate of the sludge reduction efficiency of the HS-A-OSA process in controlling excess sludge. Figure 3 shows DSP_{day} in both the experimental devices during the study period. The DSP_{day} was calculated based on equation (1) [21, 22].

$$DSP_{day} = (Q_{WAS} \times X_{WAS}) + \Delta X\ (Q_{eff} \times X_{eff}) \quad (1)$$

where Q_{WAS} is the flow rate of the waste-activated sludge in L/d, X_{WAS} is the MLSS concentration of the waste-activated sludge in g/L, ΔX is the net solids produced by devices in g/L, Q_{eff} is the effluent flow rate in L/d, and X_{eff} is the effluent MLSS concentration in g/L.

The cumulative sludge production ($DSP_{cumulative}$) was calculated based on equation (2) [21, 22].

$$DSP_{cumulative} = \sum_{day=1}^{i} DSP_{day} \quad (2)$$

The cumulative sludge production of the HS-A-OSA and A-OSA process was 477.18g and 621.26g, respectively. This production accounted for 23.2 % of sludge reduction and was calculated from the Fig. 3.

The sludge observed yields (Y_{obs}) of both experimental devices were calculated based on Eq. (3) [23–25].

$$Y_{obs} = [(Q_o - X_e) + \Delta X]/(S_o - S_e) \quad (3)$$

where X_0 is the influent suspended solids in g/L, X_e is the effluent suspended solids in g/L, ΔX is the net solids produced of devices in g/L, S_0 is the influent COD concentration in g/L, and S_e is the effluent COD concentration in g/L.

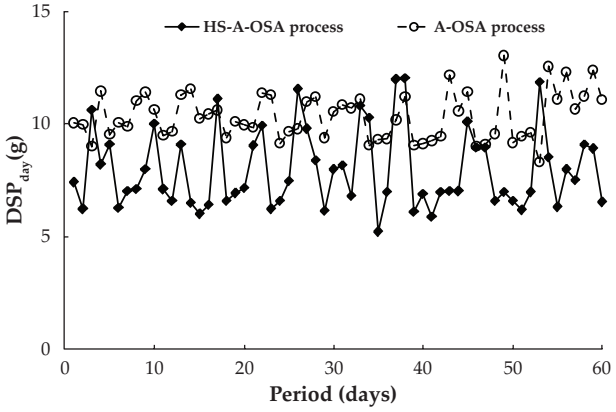


Fig. 3. DSP_{day} in different processes.

Fig. 4 shows the COD removal efficiency in both the experimental devices during the study period. The COD removal efficiencies in both the experimental devices were found to be in the ranges of 89.6%–95.0% and 87.0%–81.5%. Based on the results, it is evident that the COD removal efficiency of the HS-A-OSA process was higher than the A-OSA process.

The Y_{obs} average values for both the experimental devices were calculated to be 0.19 g MLSS/g COD and 0.25g MLSS/g COD. The cumulative sludge production and Y_{obs} showed the sludge reduction efficiency of the HS-A-OSA process was superior to the A-OSA process. The composite humic biological filler can change the composition and increase the humic acid content of the activated sludge. The common activated sludge is translated into the humic-activated sludge in the HS-A-OSA process. The humic-activated sludge returns the anaerobic sludge reaction basin of the HS-A-OSA process with nutrient deficiencies. ATP is more quickly decomposed to meet the energy demand of metabolism than the A-OSA process. When the humic sludge returns the primary reaction basins (the anoxic basin and the oxic basin) with adequate nutrition, the humic-activated sludge increases in a compensatory fashion for previous sludge attenuation. The sludge growth of the HS-A-OSA process was below the A-OSA process [26, 27]. Therefore, the sludge reduction efficiency of the HS-A-OSA process was superior to the A-OSA process.

Fig. 5 shows the TN removal efficiency in both experimental devices during the study period. The TN removal efficiencies in both experimental devices were in the ranges of 70.2–88.5% and 62.5–82.5%. Based upon the results, it is apparent that the TN removal efficiency of the HS-A-OSA process was higher than the A-OSA process.

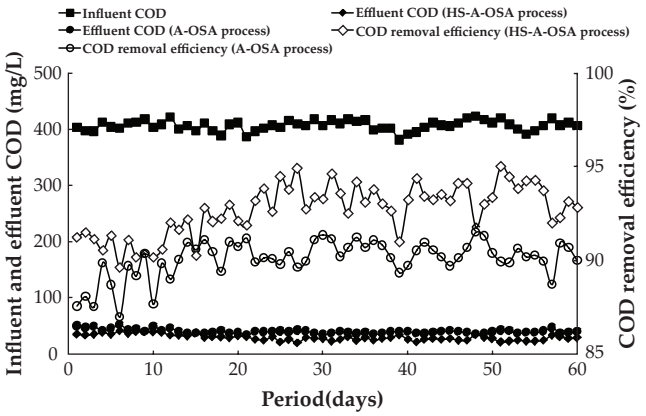


Fig. 4. COD removal efficiency in different processes.

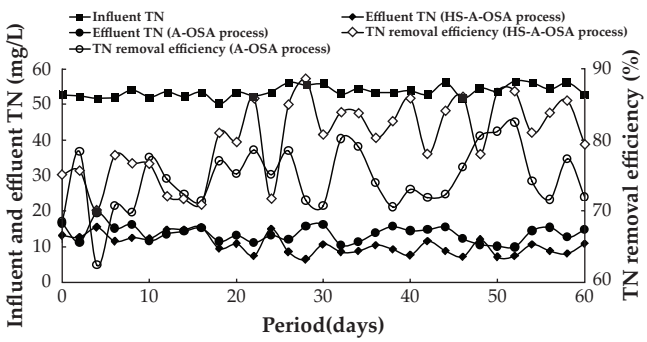


Fig. 5. TN removal efficiency in different processes.

3.2. Characteristics of humic-activated sludge

Fig. 6 shows the SVI variation in both experimental devices during the study period. The SVI in both experimental devices was in the ranges of 70.2%–88.5% and 62.5%–82.5%. Based on the results, it is clear that the sludge settling of the HS-A-OSA process was higher than the HS-A-OSA process.

Figs. 7 and 8 shows the microorganism morphologies of the primary reaction basins in both experimental devices during the study period. There was a huge amount of cocci and bacilli in the HS-A-OSA process. The microorganisms closely adhered on the surfaces of filamentous and formed compact humic-activated sludge flocs; oxidizing and settling were excellent. A portion of the cocci and bacilli attached to the filamentous surfaces were intertwined in the A-OSA process. Floc structure was comparatively looser. Therefore, the sludge settling of the HS-A-OSA process was superior to the A-OSA process.

3.3. Analysis of EPS in different reaction basins

The composite humic biological filler can releases humic substances and silicates, enrich the microorganism, and change the structure of microbial community within the activated sludge. The microorganism metabolites are influenced by the composite humic biological filler placed inside the oxic basin of the A-OSA process. Polysaccharide, protein, and humic acid are the most important components of the EPS [28]. The differences of microbial decomposition level can be detected by investigating the content of polysaccharide, protein, and humic acid in the mixed liquor of different reaction basins.

Fig. 9 shows the contents of polysaccharides, protein, humic acid, and SEPS in different reaction basins. In SEPS, the polysaccharide and protein contents of the anaerobic sludge reduction basin in the HS-A-OSA process was the highest (2.74 mg/L and 23.36 mg/L, respectively). The polysaccharide and protein contents of the primary basins in the HS-A-OSA process were lower than the A-OSA process. The main source for SEPS in the mixed liquor is

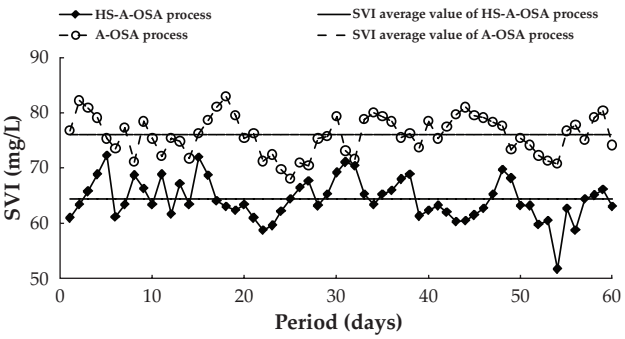
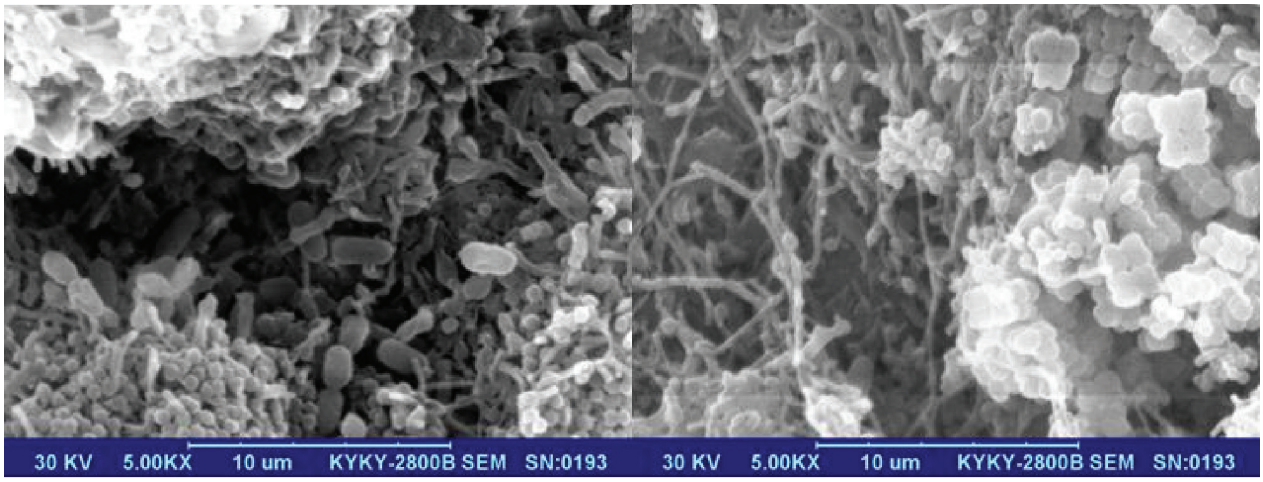
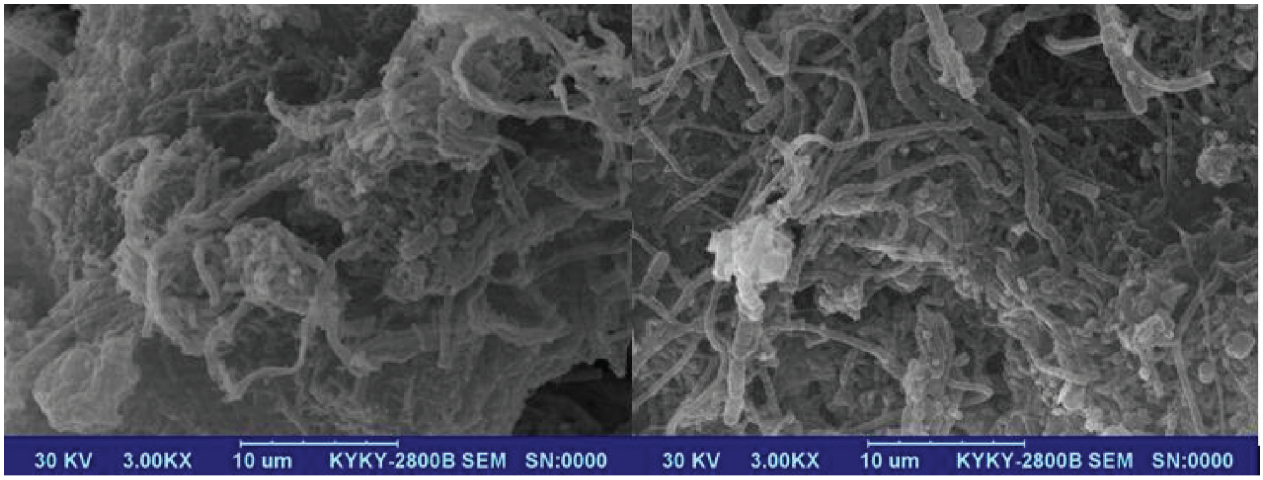


Fig. 6. SVI of oxic basins in different processes.



(a) anoxic basin (b) oxic basin

Fig. 7. SEM observations of the microorganism morphologies in HS-A-OSA process.



(a) anoxic basin (b) oxic basin

Fig. 8. SEM observations of the microorganism morphologies in A-OSA process.

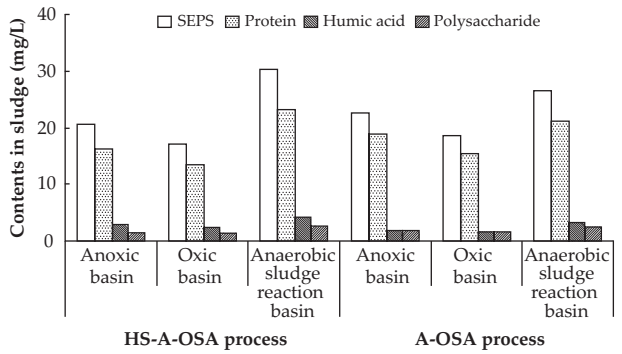


Fig. 9. Contents of polysaccharide, protein, humic acid, and SEPS in different reaction basins.

biomass decay and cell lysis. Because of the anaerobic sludge reaction basin with nutrient deficiencies, low DO, and high MLSS concentration, biomass decay and cell lysis take place. An abundance of intracellular materials contain polysaccharide and protein dissolved in water. Based on the results, it is evident that the sludge disintegration velocity of the HS-A-OSA process was higher than the A-OSA process. Therefore, the polysaccharide and protein contents of the anaerobic sludge reduction basin in the HS-A-OSA process were higher than other basins. The humic acid content of SEPS in the HS-A-OSA process was higher than the A-OSA process because the composite humic biological filler released humic substances.

Fig. 10 shows the contents of polysaccharides, protein, humic acid, and BEPS in different reaction basins. The contents of BEPS in the primary reduction basin in the HS-A-OSA process was 34.75 mg/g MLSS and 31.01 mg/g MLSS which was lower than the A-OSA process. The composite humic biological filler added inside the HS-A-OSA process may affect the microbial community and microbial metabolism, thus leading to a reduction in BEPS concentration. Because the EPS content has a positive correlation with sludge settling [29], sludge settling of the HS-A-OSA process was superior to the A-OSA process.

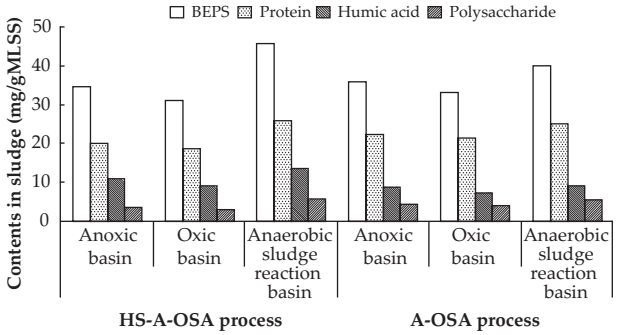


Fig. 10. Contents of polysaccharide, protein, humic acid, and BEPS in different reaction basins.

In BEPS, the polysaccharide and protein content of the anaerobic sludge reduction basin in the HS-A-OSA process was the highest (5.61 mg/g MLSS and 26.17 mg/L, respectively). When the microorganisms are in the basin with nutrient deficiencies, the principle component of EPS is polysaccharide. The disintegration velocity of the humic-activated sludge is higher, and an abundance of protein in the humic-activated sludge is released. The polysaccharide and protein contents of the anaerobic sludge reduction basin in the HS-A-OSA process were the highest. When the polysaccharide and protein returns the primary basins in the HS-A-OSA process, organic loading increases and the concentrations are lower. Organics are stored in microbial cells by saccharides in high concentrations and is involved in metabolism. The glycometabolism ability of the humic-activated sludge is better than the common activated sludge and more saccharides are turned into inorganics, while protease activity can be improved with humic acid [30]. The returned protein is largely decomposed by the humic-activated sludge in the oxic basin. The polysaccharide and protein contents of the primary basins in the HS-A-OSA process are lower than the A-OSA process. The humic acid content of BEPS in the HS-A-OSA process was higher than the A-OSA process. The main source of humic acid is the EPS of biomass decay and cell lysis, from which intracellular materials are released and adhere to the surface of activated sludge in the A-OSA process. The microbial community is optimized with the composite humic biological filler in the HS-A-OSA process. The macromolecule organics agglutinate, recombine, and humify, which is the main source of humic acid in the HS-A-OSA process. The humic acid content of basins in the HS-A-OSA process was higher than the A-OSA process.

4. Conclusion

It can be concluded that the HS-A-OSA process is possible with less cumulative sludge production when the return sludge residence time was 5.5 h in the anaerobic sludge reaction basin. Anaerobic basin with nutrient deficiencies favors biomass decay and cell lysis of the return humic-activated sludge. The disintegration of the humic-activated sludge resulted in 23.2% reduction in excess sludge. The system can run steadily with better sludge settling, COD and TN removal efficiencies. The EPS variation characteristics show the functions of the composite humic biological filler in the HS-A-OSA process.

Acknowledgments

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References

[1] Y.K. Oh, K.R. Lee, K.B. Ko, I.T. Yeom, Effects of chemical sludge disintegration on the performances of wastewater treatment by membrane bioreactor, Water Res., 41 (2007) 2665–2671.
[2] D.K. Uan, J.R. Banu, I.J. Chung, I.T. Yeom, Effect of thermochemical sludge pretreatment on sludge reduction and on performances of anoxic-aerobic membrane bioreactor treating low strength domestic wastewater, J. Che. Technol. Biotechnol., 84 (2009) 1350–1355.
[3] I. Pasztor, P. Thury, J. Pulai, Chemical oxygen demand fractions of municipal wastewater for modeling of wastewater treatment, Int. J. Environ. Sci. Technol., 6 (2009) 51–56.
[4] B. Hooshyari, A. Azimi, N. Mehrdadi, Kinetic analysis of enhanced biological phosphorus removal in a hybrid integrated fixed film activated sludge process, Int. J. Environ. Sci. Technol., 6 (2009) 149–158.
[5] R. Burkhard, A. Deletic, A. Craig, Techniques for water and wastewater management: a review of techniques and their integration in planning, Urban Water, 2 (2000) 197–221.
[6] R. Srivastava, D. Kumar, S.K. Gupta, Bioremediation of municipal sludge by vermitechnology and toxicity assessment by Allium cepa, Bioresour. Technol., 96 (2005) 1867–1871.
[7] Z. He, H.Y. Wang, H.H. Tian, L. Wang, Y.X. Zhou, Research progress in wastewater treatment processes with sludge reduction, China Water Wastewater, 25 (2009) 1–7.
[8] S. Saby, M. Djafer, G.H. Chen, Effect of low ORP in anoxic sludge zone on excess sludge production in oxic-settling-anoxic activated sludge process, Water Res., 37 (2003) 11–20.
[9] Y.H. Lu, X. Gao, Research on sludge reduction by inserting a sludge holding tank in the AO process, Muni. Eng. Technol., 26 (2008) 50–52.
[10] J. Yin, K. Zhao, Application of humus activated sludge process in Japan and the Republic of Korea, China Water Wastewater, 23 (2007) 101–104.
[11] Z.X. Liu, F.Y. Cui, S.L. Qin, L. Ding, Pollutants removal efficiency and deodorization effect in humus soil activated sludge process, China Water Wastewater, 23 (2007) 18–22.
[12] M. Wu, C.Y. Wei, X.H. Pan, R. Zhu, J.P. Sun, Y. Zhu, Study on pollutant removal efficiency of humus soil activated sludge process, China Water Wastewater, 25 (2009) 12–19.
[13] B.M. Wilén, B. Jin, P. Lant, The influence of key chemical constituents in activated sludge on surface and flocculating properties, Water Res., 37 (2003) 2127–2139.
[14] J. Zhou, H.X. Chai, T.R. Long, Study on influence factors of extra-cellular polymers in activated sludge, Water Wastewater Eng., 31 (2005) 19–23.
[15] X. Luo, Z.F. Lei, Z.Y. Zhang, S. Norio, Study on the extraction of extracellular polymeric substances (EPS) from aerobic/anaerobic sludges, Acta. Sci. Circumst., 25 (2005) 1624–1629.
[16] Ministry of Environmental Protection of the People's Republic of China, *Monitor Analysis Methods of Water and Wastewater* (4th edition), China Environment Press, Beijing 2009, pp. 210–258.
[17] B. Frølund, P. Rikke, K. Keiding, P.H. Nielsen, Extraction of extracellular polymers from activated sludge using a cation exchange resin, Water Sci. Technol., 30 (1996) 1749–1758.
[18] B. Frølund, T. Griebe, P.H. Nielsen, Enzymatic activity in the activated sludge floc matrix, Appl. Microbiol. Biotechnol., 43 (1995) 755–761.
[19] G.H. Yu, P.J. He, L.M. Shao, Y.S. Zhu, Extracellular proteins, polysaccharides and enzymes impact on sludge aerobic digestion after ultrasonic pretreatment, Water Res., 42 (2008) 1925–1934.
[20] Y.C. Chiu, C.N. Chang, J.G. Lin, S.J. Huang, Alkaline and ultrasonic pretreatment of sludge before anaerobic digestion, Water Sci. Technol., 36 (1997) 155–162.

[21] D.K. Uan, J.R. Banu, D.H. Son, I.T. Yeom, Influence of ferrous sulfate on thermochemical sludge disintegration and on performances of wastewater treatment in a new process: Anoxic–oxic membrane bioreactor coupled with sludge disintegration step, Biochem. Eng. J., 66 (2012) 20–26.
[22] J.R. Banu, S. Kaliappan, A. Kumar, I.T. Yeom, D.K. Uan, Effect of low temperature thermochemical pretreatment on sludge reduction potential of membrane bioreactor treating primary treated dairy wastewater, Water Qual. Res. J. Can., 46 (2011) 312–320.
[23] J.R. Banu, D.K. Uan, I.T. Yeom, Nutrient removal in an A^2O-MBR reactor with sludge reduction, Bioresour. Technol., 100 (2009) 3820–3824.
[24] J.R. Banu, D.K. Uan, S. Kaliappan, I.T. Yeom, Effect of sludge pretreatment on the performance of anaerobic/anoxic/oxic membrane bioreactor treating domestic wastewater, Int. J. Environ. Sci. Technol., 8 (2011) 281–290.
[25] M.S.K. Kumar, T.K. Kumar, P. Arulazhagan, S.A. Kumara, I.T. Yeom, J.R. Banu, Effect of alkaline and ozone pretreatment on sludge reduction potential of a membrane bioreactor treating high-strength domestic wastewater, Desal. Wat. Treat., 55 (2015) 1127–1134.
[26] Z.Y. Yuan, The Analysis of EPS and Sludge Characteristics under Different Working Conditions in the IBR, Huazhong University of Science and Technology, Wuhan, 2011.
[27] G.H. Chen, K.J. An, S. Saby, E. Brois, M. Djafer, Possible cause of excess sludge reduction in an oxic-settling-anaerobic activated sludge process (OSA process), Water Res., 37 (2003) 3855–3866.
[28] W.Y. Li, J.J. Xu, M. Wu, H. Lu, Improvement of humus soil on activated sludge properties under condition of thermophilic anaerobic digestion, J. Tongji Univ., 40 (2012) 1349–1356.
[29] H. Liu, H.H. Fang. Extraction of extra cellular polymeric substances (EPS) of sludges, J. Biotechnol., 95 (2002) 249–256.
[30] R.X. Wang, Humic acid and soil enzyme activity, Humic Acid, 3 (1998) 27–28.